TABLE 3 Representative microprobe analyses of eruptive products

		SiO_2	Al_2O_3	FeO	MgO	CaO	Na_2O	K_2O	TiO_2	MnO	Cr_2O_3	NiO	Total	Mg#
7-1-91-1a 12 June 1991 andesite scoria														
ol		40.81	—	12.36	47.51	0.12	—	—	—	0.13	—	0.09	101.13	87.1
sp(ol)		0.25	16.22	33.75	10.88	—	—	—	1.11	0.11	35.52		97.83	52.1
ag		51.69	2.86	5.24	16.11	22.29	0.14	—	0.45	0.04	0.22	—	99.04	84.4
gl	mean (n=12)	70.39	14.96	2.41	0.72	2.57	2.81	3.18	0.31	—	—		97.37	
gl	s.t.d.	1.18	0.70	0.14	0.16	0.16	0.56	0.17	0.05				0.97	
EW910615-1 15 June 1991 Crystal-rich pumice (white)														
cm		54.59	1.64	16.72	21.30	1.77	0.16	—	0.12	0.79	—	—	97.11	68.4
hb		48.26	7.27	13.02	15.19	10.69	1.16	0.26	0.78	0.49	—	—	97.14	66.7
gl	mean (n=16)	76.55	12.47	0.75	0.10	1.20	3.52	3.07	0.06	—	—		97.72	
gl	s.t.d.	0.78	0.23	0.03	0.02	0.04	0.72	0.24	0.03				0.85	
EW910615-2 15 June 1991 Crystal-poor pumice (tan)														
cm		54.88	1.25	16.92	21.41	1.39	0.07	—	0.11	1.06	—	—	97.08	68.0
hb		48.13	7.39	13.12	15.48	10.24	1.24	0.20	0.93	0.43	—	—	97.12	67.1
gl	mean (n=6)	73.69	13.34	1.39	0.62	2.22	2.85	2.80	0.16	—	—		97.06	
gl	s.t.d.	3.27	1.26	0.87	0.72	1.50	0.55	0.48	0.13				1.08	
PH13-D 15 June 1991 Crystal-poor pumice (tan)														
gl	mean (n=5)	67.93	15.18	3.22	2.77	4.49	3.05	2.01	0.29	—	—		98.95	
gl	s.t.d.	0.79	0.54	0.25	0.71	0.52	0.87	0.17	0.04				1.42	

A dash indicates that the oxide was undetected or near detection limit (~0.05%). Abbreviations: ol, olivine; sp(ol), spinel in olivine; ag, augite; gl, glass; cm, cummingtonite. Analysed at 15 keV, 15 nA sample current (on brass). Spot size 20 μm on phenocrysts, point beam on microlites and glass bubble walls. Glass analyses corrected for migration of Na, K, Si and Al using iterative 1-s count data. Mg# = Mg/(Mg + Fe + Mn) × 100 (equivalent to forsterite content of olivine). Total iron reported as FeO, except for spinels, which are recalculated assuming $R^{2+}R^{3+}_2O_4$ formulae and the sum of FeO and Fe_2O_3 reported.

in the thermal boundary layer of 20–30 vol% to explain the difference in crystal contents of the crystal-rich and crystal-poor pumice types (Table 1). A minimum volume of 1 km^3 for the boundary layer is estimated from the proportion of crystal-poor dacite (20%) in the inferred erupted magma volume of ~5 km^3 (ref. 14). Assuming a ratio of dacite melted to basalt crystallized of ~0.4 (ref. 15), a minimum of ~1/2 to 3/4 km^3 of basalt crystallization is required, so a much larger volume of basaltic magma must have been present to allow magma mixing. Involvement of a relatively large volume of basalt is also suggested by the large size of the magma chamber inferred from seismic data[16]. Preliminary analysis of the distribution of earthquake hypocentres that accompanied the Pinatubo eruptions suggests a magma body that extends from ~5 to >15 km with a volume of >50 km^3.

The basaltic underpinnings of the magma reservoir may have contributed to the 20 megatons of SO_2 (ref. 17) emitted to the atmosphere during the Pinatubo eruptions. Given abundances of 500–1,000 p.p.m. sulphur in basaltic magmas[18], degassing of a relatively large volume of basalt (4–8 km^3) would be required for mass balance of the emissions. The origin of the atmospheric SO_2 is currently attributed either to degassing of an exsolved fluid phase in the dacite[19], or to breakdown of anhydrite[11]. We suggest that the ultimate source for the sulphur could lie at greater depth. The dacite may have been enriched in sulphur through upward migration of a fluid phase derived from basaltic magma at deeper levels of the volcano's feeder system.

Note added in proof: Andesite blocks derived from the 7–11 June 1991 lava dome contain abundant basaltic inclusions and provide strong support for the magma mixing model proposed here. A quenched inclusion from a sample provided by C. G. Newhall is magnesian (Mg# = 68) dictytaxitic basalt with phenocrysts of olivine (Fo_{84-89}, rimmed by hornblende), augite and hornblende. Least-squares modelling of major-element data shows that a 40:60 mixture of this basalt and the crystal-rich dacite would produce mixed andesite that is virtually identical to the 12 June scoria. □

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1. Anderson, A. T. *J. Volcanol. geotherm. Res.* **1,** 3–33 (1976).
2. Sakuyama, M. *J. Volcanol. geotherm. Res.* **5,** 179–208 (1979).
3. Sparks, R. S. J., Sigurdsson, H. & Wilson, L. *Nature* **267,** 315–318 (1977).
4. Hoblitt, R. P. *et al. Trans. Am. geophys. Un.* **72,** 61 (1991).
5. Roeder, P. L. & Emslie, R. F. *Contrib. Miner. Petrol.* **29,** 275–289 (1970).
6. Ulmer, P. *Contrib. Miner. Petrol.* **101,** 261–273 (1989).
7. Fournelle, J. H. *Trans. Am. geophys. Un.* **72,** 68 (1991).
8. Wolfe, J. A. & Self, S. *Phil. J. Volcanol.* **1,** 11–40 (1983).
9. Bernard, A., Demaiffe, D., Mattielli, N. & Punongbayan, R. S. *Nature* **354,** 139–140 (1991).
10. Melson, W. G. *Science* **221,** 1387–1391 (1983).
11. Rutherford, M. J. & Devine, J. D. *Trans. Am. geophys. Un.* **72,** 62 (1991).
12. Huppert, H. E. & Sparks, R. S. J. *J. Petrol.* **29,** 599–624 (1988).
13. Eichelberger, J. C. *Nature* **288,** 446–450 (1980).
14. Scott, W. E., Hoblitt, R. P., Daligdig, J. A., Besana, G. & Tubianosa, B. S. *Trans. Am. geophys. Un.* **72,** 61–62 (1991).
15. Bergantz, G. W. *Science* **245,** 1093–1095 (1989).
16. Mori, J. *et al. Trans. Am. geophys. Un.* **72,** 62 (1991).
17. Bluth, G. J. S., Doiron, S. D., Krueger, A. J., Walter, L. S. & Schnetzler, C. C. *Geophys. Res. Lett.* (in the press).
18. Devine, J. D., Sigurdsson, H. & Davis, A. N. *J. geophys. Res.* **89,** 6309–6325 (1984).
19. Westrich, H. R. & Gerlach, T. M. *Trans. Am. geophys. Un.* **72,** 62 (1991).

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The fungus *Armillaria bulbosa* is among the largest and oldest living organisms

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ASEXUALLY reproducing organisms occur in a variety of taxa in all biological kingdoms[1] and distinguishing asexually propagated genotypes is essential for the understanding of their population biology. Among the higher fungi, however, the clonal 'individual' is especially difficult to define[2] because most of the fungal thallus consists of a network of anastamosing hyphae embedded in the substratum. Whether fruit-bodies, the most recognizable part of a fungus, are produced by a single supporting mycelium can only be determined by establishing direct physiological continuity or genetic identity. We report a means by which individual fungi can be unambiguously identified within local populations and identify an individual of *Armillaria bulbosa* that occupies a minimum of 15 hectares, weighs in excess of 10,000 kg, and has remained genetically stable for more than 1,500 years.

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TABLE 1 Distribution of genetic markers in clone 1 monosporous isolates

	Monosporous isolate*															
	438-				445-				454-				455-			
	1	2	8	9	3	4	6	10	2	4	7	9	5	8	9	10
MATA	1	2	2	1	1	2	1	2	1	2	2	1	1	2	1	2
MATB	1	1	2	2	1	1	2	2	1	2	1	2	2	1	1	2
pVII-8	1	1	1	2	2	1	2	1	1	1	1	1	2	2	1	2
pVII-13	2	2	1	1	1	1	1	2	2	1	2	2	2	1	2	2
pVII-18	1	1	1	1	2	2	2	1	2	2	1	2	2	2	2	2
pVII-23	1	1	2	2	2	2	2	1	1	2	1	1	1	2	1	1
pJA1	1	2	2	2	1	2	2	1	2	2	1	1	1	1	1	1
R25-1	+	+	+	0	+	+	0	+	+	0	+	0	+	+	0	+
R25-2	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+
R25-3	+	0	+	0	0	+	+	0	0	0	+	+	0	+	+	0
R25-4	+	0	+	+	+	+	+	+	0	0	+	+	+	+	+	+
R25-5	+	+	0	+	0	0	0	0	+	+	0	0	0	0	0	0
R25-6	0	+	+	+	0	+	+	0	+	+	0	0	0	0	0	0
R28-1	+	+	0	+	+	+	0	+	+	0	+	0	+	0	0	+
R28-2	0	+	+	0	0	+	0	0	0	+	0	0	0	0	0	0
R32-1	+	+	+	0	+	0	0	0	0	+	+	0	+	+	+	+
R32-2	0	+	+	+	0	+	+	0	+	+	0	0	+	+	+	+
R32-3	0	0	0	+	0	0	+	+	0	+	+	+	0	0	0	0

Each data set includes the four possible mating-types (*MATA*, *MATB*) segregating at meiosis from four fruit-bodies of clone 1. RFLP probes (pVII-8, -13, -18, -23) are anonymous nuclear DNA *Eco*RI fragments cloned in pUC18 from an *A. bulbosa* isolate by standard methods[27]; pJA1 contains a portion of the nuclear rDNA repeat of *A. ostovae*[28]. Of several RAPD primers tested, three gave multiple fragments: primer R25 (ACTTGAGGCG) was one of a series of arbitrary primer sequences that produced six, and primer R28 (ATGGATCCGC) produced two segregating fragments; primer R32 (CATCATCATCATCAT) is a repeated sequence found in a wide range of organisms (GenBank) and in the monosporous isolates examined produced 3 segregating fragments. RAPD fragments marking heterozygous loci segregated consistently in three independent experiments as present (+) or absent (0), and therefore act as dominant markers. To avoid bias, we chose marked loci that were not closely linked to one another, nor to either mating-type locus, which are always heterozygous. Allele designations are arbitrarily assigned for each RFLP, RAPD and mating-type locus.

* First number denotes fruit-body, second number denotes monospores isolate (see Fig. 1).

A. bulbosa is a facultative tree-root pathogen, commonly found in European and eastern North American mixed hardwood forests[3,4]. It does not produce conidia or any other vegetative propagules but thalli form abundant macroscopic cord-like aggregations of hyphae, called rhizomorphs, which facilitate vegetative spread and food base acquisition in the forest floor.

Previous attempts to determine the size of individual clones of basidiomycetes have relied on two criteria: assay of somatic interactions[5-7] and distribution of mating-type alleles[8,9]. Somatic interactions suggest genetic dissimilarity when an 'antagonistic' reaction line develops along the contact zone between two isolates growing together, whereas isolates of the same or similar genetic constitution seem to mingle freely and establish a stable cytoplasmic continuum. The second method uses the bifactorial sexual incompatibility system found in most basidiomycetes including *A. bulbosa*[10], in which compatible matings occur only when paired monosporous isolates have different alleles at two unlinked, multiallelic loci, designated *A* and *B* (ref. 11). It is then assumed that fruit-bodies with the same alleles at *A* and *B* loci and growing in 'reasonable' proximity are from the same diploid vegetative mycelium.

Using these criteria, it has been inferred that basidiomycete clones can exceed 500 m in diameter[7,12,13], which is surprising as mushrooms are perceived as spatially discontinuous and ephemeral. Unfortunately, neither method distinguishes a single large clone from a number of closely related individuals. As spore deposition decreases rapidly from the point of liberation[14], mating between compatible spore offspring would generate a cluster of individuals that are genetically distinct but share the same four parental mating-type alleles. Furthermore, about half of such sib-related individuals may not be somatically antagonistic[8,13]. To discriminate clearly between vegetative growth of an individual and multiple matings involving similar genotypes, we examined allelic composition at heterozygous loci marked by restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs) (ref. 15) in *A. bulbosa*.

In September of 1988 to 1991, *A. bulbosa* isolates (species VII; ref. 3) were obtained from fruit-bodies and rhizomorphs along 1 km east/west, and north/south transects through a permanent study site in a northern Michigan hardwood forest. A region of 15 hectares yielded *A. bulbosa* isolates with identical mating-type alleles and mitochondrial DNA restriction fragment patterns, both of which are highly polymorphic within the species[16]. We provisionally refer to this group of collections as 'clone 1' (Fig. 1). If clone 1 is made up of inbred siblings, about half would show loss of heterozygosity at any unconstrained genetic locus that is heterozygous in the parent. In fact both allelic forms of each of five loci marked by RFLPs (Table 1) were clearly evident in Southern blot analyses of genomic DNAs from representative vegetative (diploid) isolates throughout clone 1. In addition, all 11 RAPD products, each marking a heterozygous locus (Table 1), were present in all vegetative isolates (Fig. 2).

The probability of retaining heterozygosity at each parental locus in an individual produced by a mating of sibling monosporous isolates is $P = (0.5)^m(0.75)^n$, where m is the number of co-dominant (RFLP) loci and n is the number of loci at which one allele is dominant (RAPD). In the case of clone 1, $P = (0.5)^5(0.75)^{11} = 0.0013$. This low probability allows rejection of the hypothesis that the clone-1 isolates arose through independent matings. Indeed, the corresponding probability for either the 5 RFLP or the 11 RAPD markers taken separately, is <0.05 in each case. There is no obvious constraint toward heterozygosity at any of these loci unlike mating-type loci. Monosporous isolates showed normal ability to mate and produce homozygous mycelia in laboratory crosses. In addition, a neighbouring *A. bulbosa* clone (clone 2, Fig. 1) was homozygous at two of the RFLP loci, and lacked five of the clone 1 heterozygous RAPD fragments, implying homozygosity at these loci. Although the genetic markers used here are probably selectively neutral, it was not our goal to assess correlations of fitness and heterozygosity under natural conditions. The most acceptable remaining hypothesis is that clone 1 reached its present size through vegetative growth. As each clone occupies a discrete, contiguous territory and apparently excludes other clones of the same

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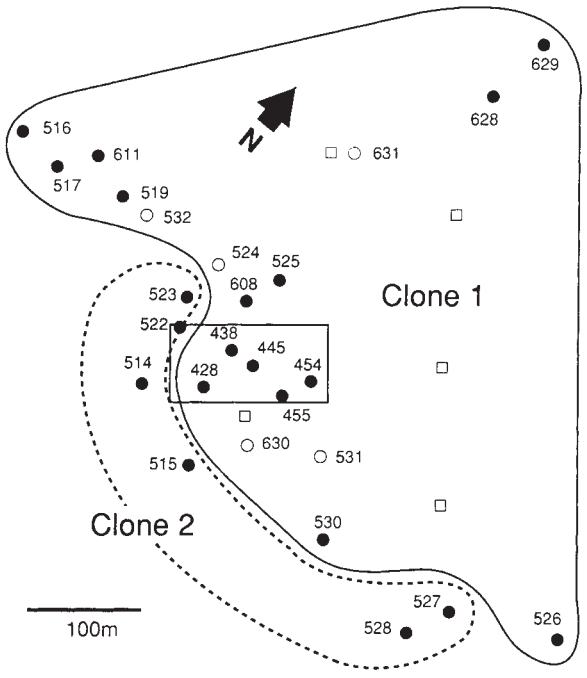

FIG. 1 Size and distribution of two *A. bulbosa* clones within a 30 hectare site. Cultures obtained from rhizomorph samples (608, 611, 628, 629) and subhymenium of fruit-bodies (428, 454, 516, 517, 519, 525, 526, 530) were chosen for genetic analysis to represent vegetative, diploid isolates throughout 15 hectare 'clone 1' (solid line). Heterozygous loci (Table 1) were assayed in monosporous isolates from fruit-bodies 438, 445, 454 and 455. Additional *A. bulbosa* isolates (open circles) had clone-1 mating-type alleles and/or mitochondrial DNA type. No overlap of clone 1 and clone 2 (dashed line) was observed within an intensively sampled 0.8 hectare area (37 sample points within blocked area[16]). Linear growth rates of clone 1 rhizomorphs were determined by three independent methods: (1) rhizomorph growth along 45 poplar stakes buried within clone 1, measured after one growing season. The mean (±s.d.) maximum length of the ten longest was 19.7 ± 3.1 cm; (2) maximum rhizomorph growth rates for clone 1 isolates on Weinhold's media with 0.24 g l^{-1} ethanol to enhance rhizomorph growth[25], at 8, 12, 15, 22 and 28 °C, were 0.0, 0.0, 2.1, 4.7, and 9.0 mm d^{-1}, respectively; (3) colonized 160 cm^3 wood blocks with rhizomorph initials were placed in flats of topsoil taken from the study site and incubated at 10, 15 and 22 °C for 40 d. Respective mean maximum growth rates under these conditions were 0.2 ± 0.2, 0.3 ± 0.3, and 2.2 ± 0.4 mm d^{-1} (± s.d., $n = 6$ for each temperature). Soil temperature at the study site approaches 14 °C at 10 cm depth and 14–16 °C at lesser depths for about 90 d per year. The annual growth rate of clone 1 is therefore estimated to be 20 cm by methods 1 and 2, and ≤6 cm by method 3. These are low compared to rhizomorph growth rates reported for *Armillaria* species[6,29,30], in keeping with a short growing season and heavy, prolonged snow pack in the area. To estimate the mass of clone 1 rhizomorphs 25 soil pits measuring (15 cm)^3 were taken within clone 1 (open squares) and dissected to separate rhizomorphs from fine roots. The average dry weight of rhizomorphs from all soil pits was 0.1406 ± 0.1400 g per 225 cm^2, or about 6.25 g m^{-2}. This dry weight measure is equal to a fresh rhizomorph weight of 64.4 g m^{-2} and mean rhizomorph length of 17 m per m^2 close to abundance estimates in other places[26].

species, dispersal of rhizomorphs, fruit-bodies or other mycelial fragments at the study site is unlikely. Other fungi that do produce vegetative propagules, exhibit an intermingling, mosaic pattern of genotypes in local areas[17,18].

Identifying 'clone 1' as a single genetic individual enables us to make minimal estimates of its age. Rhizomorph growth rates for clone 1 isolates were consistently ≤0.2 m per year (Fig. 1 legend). Estimating clone 1 to be 635 m across, its age is therefore about 1,500 years. This is probably an underestimate as antagonistic interactions would impede extension into areas occupied by other *A. bulbosa* clones. Clearly, clone 1 was established before the standing 60-year-old forest, and before a 300-year-old white pine/mixed hardwood forest that existed at the site before a 1928 fire. *Armillaria* rhizomorphs are known to withstand above-ground fires of extremely high temperatures[19].

Given its age, the genetic stability of clone 1 is remarkable. In our study of heterozygous loci, a total of 20 RAPD and 27 nuclear DNA restriction fragments (including those in Table 1) were invariate in all diploid isolates of clone 1. This constitutes a direct measure of about 0.73 kilobases of nucleotide sequence (20 base pairs for each RAPD product; 12 base pairs for each *Eco*RI fragment assayed) and indicates the absence of DNA sequence rearrangements, insertions, deletions and other internal RFLP and RAPD site gains, which would be detected as polymorphisms. Our data also show no evidence for mitotic crossing over[20] with its attendant loss of heterozygosity, or for mutation.

Determination of reproductive success has not been made for any fungal individual because of the difficulties in tracking the dispersal and establishment of windborn spores[21]. As more than 10 *Armillaria* fruit-bodies can occur (on average) in a 5 m^2 area and a single basidiomycete fruit-body can distribute 1,000,000 spores per hour over several days[22], the potential reproductive output of an individual the size and age of clone 1 is enormous. The successful colonization of areas already occupied by other

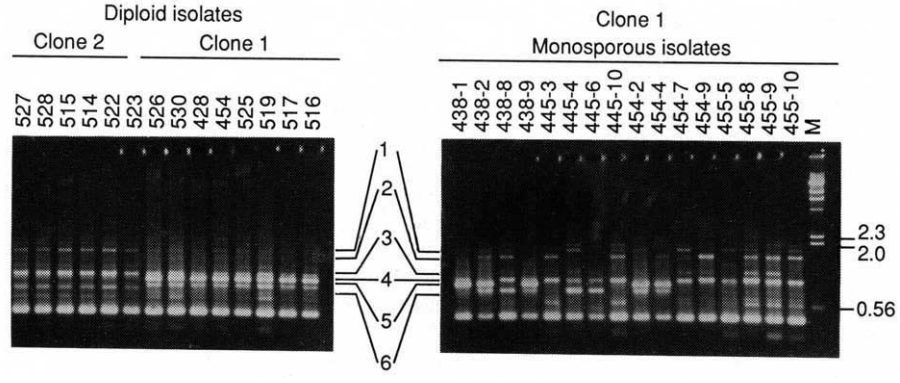

FIG. 2 RAPD products from primer R25 for monosporous isolates of clone 1 and diploid isolates of clones 1 and 2. Six fragments segregating for presence or absence in monosporous isolates are indicated in the centre, molecular size standards (lane M, in kb) are to the right.
METHODS. Polymerase chain reactions of 25 μl volume included roughly 10 ng genomic DNA with 200 μM each of dATP, dTTP, dGTP and dCTP, 1.0 μM primer oligonucleotide (Regional DNA Synthesis Laboratory, Calgary, Alberta), and 2.5 μl 10 × buffer with 0.75 units *Taq* DNA polymerase (Bio/Can, Ontario). Reactions used an initial 3-min denaturation at 93 °C, followed by 35 cycles of 37 °C for 1 min, annealing; 71 °C for 1 min, polymerization; 93 °C for 1 min, denaturation, in an M.J. Research FF100 Programmable Thermal Controller. Electrophoresis of 12-μl post-reaction samples was in 1.5% agarose, 1 × TAE buffer[27] before ethidium bromide staining and photography over a longwave ultraviolet light source.

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clones of *A. bulbosa*, however, must be vanishingly rare. We observed rhizomorphs on 88 of 123 poplar stakes (72%) from a 130-m transect through clone 1 after one year, showing that new food bases are quickly colonized by the established clone.

Evidently, individuals of *A. bulbosa* are a substantial component of mixed oak forests. At the Michigan site, the fresh weight of clone 1 rhizomorphs in soil is estimated to be about 9,700 kg (Fig. 1 legend). This estimate does not include rhizomorphs below 15 cm, nor those associated with tree roots or other wood. Significantly, this value does not include the mass of fine hyphae in the soil and wood that necessarily supports rhizomorph growth. Depending on nutrient culture conditions[23,24], *Armillaria* hyphal mass may exceed rhizomorph mass 50-fold. In agar amended with hot-water extracts of forest soil, this ratio is 10.7 ± 4.4 ($\pm$s.d., $n = 5$). In addition, *A. bulbosa* may occupy substantial volumes of suppressed or dead tree roots in oak forests[25], within which mycelia predominate. A conservative minimum mass estimate of the organism might therefore be 10 times that of the rhizomorphs, or, in the case of clone 1, about 100 tonnes, close to the mass of an adult blue whale. By comparison, *Sequoiadendron giganteum*, the largest plant on earth, attains a mass of over 1,000 tonnes, most of which is non-living wood that has accumulated during thousands of years of growth. In contrast to these more highly integrated organisms, fungi may undergo dramatic fluctuations in mass, for example, up to threefold within a few years, depending on recent forest history[26]. This is the first report estimating the minimum size, mass and age of an unambiguously defined fungal individual. Although the number of observations for giant plants and animals is much greater, members of the fungal kingdom should now be recognized as among the oldest and largest organisms on earth. □

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1. Jackson, J. B. C., Buss, L. W. & Cook, R. E. (eds) *Population Biology and Evolution of Clonal Organisms* (Yale Univ. Press, New Haven and London, 1985).
2. Rayner, A. D. M. *Mycologia* **83,** 48–71 (1991).
3. Motta, J. J. & Korhonen, K. *Mycologia* **78,** 471–474 (1986).
4. Roll-Hansen, F. *Eur. J. For. Path.* **15,** 22–31 (1985).
5. Adams, D. H. *Northw. Sci.* **48,** 21–28 (1974).
6. Shaw, C. G. III, & Roth, L. F. *Phytopathology* **66,** 1210–1213 (1976).
7. Dickman, A. & Cook, S. *Can. J. Bot.* **67,** 2005–2016 (1989).
8. Korhonen, K. *Karstenia* **18,** 31–42 (1978).
9. Ullrich, R. C. & Anderson, J. B. *Exp. Mycol.* **2,** 119–129 (1978).
10. Guillaumin, J.-J., Anderson, J. B. & Korhonen, K. in *Armillaria Root Disease* (eds Shaw, C. G. III & Kile, G. A.) 10–20 (Agricultural Handbook No. 691, US Department of Agriculture, Washington DC, 1991).
11. Raper, J. R. *Genetics of Sexuality in Higher Fungi* (Ronald, New York, 1966).
12. Anderson, J. B., Ullrich, R. C., Roth, L. F. & Filip, G. M. *Phytopathology* **69,** 1109–1111 (1979).
13. Kile, G. A. *Aust. J. Bot.* **31,** 657–671 (1983).
14. Ingold, C. T. *Fungal Spores, Their Liberation and Dispersal* (Clarendon, Oxford, 1971).
15. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. *Nucleic Acids Res.* **18,** 6531–6535 (1990).
16. Smith, M. L., Duchesne, L. C., Bruhn, J. N. & Anderson, J. B. *Genetics* **126,** 575–582 (1990).
17. Kohn, L. M., Stasovski, E., Carbone, I., Royer, J. & Anderson, J. B. *Phytopathology* **81,** 480–485 (1991).
18. McDonald, B. A. & Martinez, J. P. *Phytopathology* **80,** 1368–1373 (1990).
19. Hood, I. A. & Sandberg, C. J. in *Proc. 7th Int. Conf. on Root and Butt Rots* (ed. Morrison, D. J.) 288–296 (Ministry of Supply and Services, Victoria, British Columbia, 1989).
20. Frankel, C. *Genetics* **92,** 1121–1126 (1979).
21. Adams, T. J. H., Williams, E. N. D., Todd, N. K. & Rayner, A. D. M. *Trans. Br. mycol. Soc.* **82,** 359–361 (1984).
22. Buller, A. H. R. *Researches on Fungi Vol. 1* (Longmans, Green, London, 1909).
23. Moody, A. R. & Weinhold, A. R. *Phytopathology* **62,** 264–267 (1972).
24. Weinhold, A. R. & Garraway, M. O. *Phytopathology* **56,** 108–112 (1966).
25. Thompson, W. & Boddy, L. *New Phytol.* **93,** 277–291 (1982).
26. Twery, M. J., Mason, G. N., Wargo, P. M. & Gottschalk, K. W. *Can. J. For. Res.* **20,** 674–678 (1990).
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
28. Anderson, J. B., Bailey, S. S. & Pukkila, P. J. *Evolution* **43,** 1652–1662 (1989).
29. Rishbeth, J. *Trans. Br. mycol. Soc.* **51,** 575–586 (1968).
30. Kable, P. F. *Trans. Br. mycol. Soc.* **62,** 89–98 (1974).

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Polymorphism in red photopigment underlies variation in colour matching

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GENETIC variation of human senses within the normal range probably exists but usually cannot be investigated in detail for lack of appropriate methods. The study of subtle perceptual differences in red–green colour vision is feasible since both photopigment genotypes and psychophysical phenotypes can be assessed by sophisticated techniques. Red–green colour vision in humans is mediated by two different visual pigments: red (long-wavelength sensitive) and green (middle-wavelength sensitive). The apoproteins of these highly homologous photopigments are encoded by genes on the X chromosome[1]. Colour matches of males with normal colour vision fall into two main groups that appear to be transmitted by X-linked inheritance[2–6]. This difference in colour matching is likely to reflect small variations in the absorption maxima of visual pigments[7–11], suggesting the presence of two common variants of the red and/or green visual pigments that differ in spectral positioning[5,6]. We report that a common single amino-acid polymorphism (62% Ser, 38% Ala) at residue 180 of the X-linked red visual pigment explains the finding of two major groups in the distribution of colour matching among males with normal colour vision.

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Fifty young Caucasian males with normal colour vision were recruited for this study. The determination of Rayleigh colour matches followed an algorithm[12] that measured the proportion of red (667 nm) in a mixture of red and green (551 nm) that was perceived as identical to a standard yellow (590 nm) light. The ranges of red/green mixtures accepted by subjects as matching the yellow comparison light, as well as the match midpoints (centres of match ranges) fell within the limits that define normal observers but were consistently different among observers. Some individuals clearly required more red whereas others required more green light to match the yellow comparison light.

The gross structure and coding sequences of the red and green pigment genes of each individual were determined by Southern blot[1,13] and single-strand conformation polymorphism (SSCP) analyses followed by sequencing of polymerase chain reaction (PCR)-amplified DNA segments (Fig. 1). The results show that each subject had one red pigment gene and one or more green pigment genes as previously described[1,13]. In addition, red and green opsins are both found to be highly polymorphic. A common polymorphism was observed at amino acid residue 180 of red opsin; 31 (62%) subjects had Ser (TCT), while 19 (38%) had Ala (GCT), indicating that two major forms of red opsins exist in the general population (Table 1), a possibility earlier surmised from microspectrophotometric measurements[14]. This Ser/Ala polymorphism was observed previously when the red opsin sequences of 3 individuals were determined[1]. A frequency distribution histogram of the match midpoints for subjects with Ala or Ser at position 180 of red opsin is shown in Fig. 2. Higher sensitivity to red light (shown by a lower match midpoint in Fig. 2) is strongly correlated with the presence of Ser (Table 1).

Further evidence that the Ser/Ala polymorphism in red opsin is responsible for the variation in colour matching was obtained by studying nine deuteranopes who had no green pigment genes. Five of these individuals had Ser and four had Ala at position 180. The results were consistent with those in colour-normal

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